

Sensitivity, Specificity, and Predictive Values of Three *Salmonella* Rapid Detection Kits Using Fresh and Frozen Poultry Environmental Samples versus Those of Standard Plating

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To reduce human exposure to *Salmonella* spp. in poultry products, broiler chicken flocks have been tested by culture methods. Since the standard techniques may take 3 to 5 days, rapid detection methods have been developed. In this study we tested the performance of three rapid tests originally developed for food samples by using environmental samples obtained from poultry houses. These rapid tests were Reveal, an enzyme-linked immunosorbent assay from Neogen Corp.; BIND, a bacterial ice nucleation detection method from Idetek Corp.; and a filter monitor method from Future Medical Technologies, Inc. For the standard culture, brilliant green with novobiocin and xylose–lysine–tergitol-4 agar were used for presumptive identification, and identities were confirmed by using poly-O antisera. Environmental samples were collected from farms belonging to an integrated poultry company prior to chick placement and 1 week before slaughter. Sensitivities, specificities, and predictive values with 95% confidence intervals were calculated. Statistical differences were determined by using McNemar's chi square test. The sensitivities of the different tests were not stable, varying widely between sample times, and were affected by freezing of the samples. All of the rapid tests had low sensitivities, which led to many false-negative results. All tests were able to detect *Salmonella* spp. at a concentration of 10 CFU/ml in at least one of four trials. The BIND and Reveal tests were simple to use with multiple samples and reduced laboratory time by up to 1 day. Based on our results, we do not recommend that any of these rapid tests, in their present state of development, be utilized with environmental samples collected with drag swabs.

Poultry and poultry products have been implicated as a major source of *Salmonella* infection in humans. The estimated costs of human food-borne illness (including medical treatment, lost wages, and death) in the United States range from \$8.5 billion to \$20 billion annually (19). As a consequence, pathogen reduction control programs, such as the Hazard Analysis of Control Points program, have been developed to assess and reduce pathogen contamination of poultry products. Currently, the standard culture techniques for *Salmonella* spp. may take 3 to 5 days to determine if a sample is positive or negative. Growers could be burdened with the extra cost of holding birds to confirm a flock's *Salmonella* status. Recall of a poultry product could occur if it is found to be contaminated after it has left the processing plant. In an attempt to reduce the time and expense necessary for conventional culture techniques, many rapid detection methods have been developed as screening tests for *Salmonella* spp. Some of these methods include semisolid media, impedimetric techniques, enrichment serology, fluorescent antibody techniques, enzyme-linked immunosorbent assays (ELISA), immunodiffusion, and DNA hybridization (2). Several test kits are commercially available

for use in food safety laboratories (2, 9, 21). Many of these techniques may reduce the time needed to get results by 1 to 5 days (2, 3, 20). The majority of the methods currently available for use in food safety laboratories were developed for use with food samples, not environmental samples (2, 9, 21).

The aim of this study was to evaluate how three rapid tests that were originally developed for food samples performed with environmental samples (fresh and frozen) obtained from poultry houses. Our ultimate goal was to determine whether these rapid tests could be incorporated into preharvest control efforts. Thus, we compared the sensitivities, specificities, and predictive values of the three rapid tests to those of a standard culture technique. Lastly, we determined the minimum number of CFU necessary to label an environmental sample *Salmonella* positive with the standard culture method and each rapid method. The rapid tests used were Reveal, a colloidal gold-labeled antibody ELISA from Neogen Corp.; BIND, a bacterial ice nucleation detection test from Idetek Corp.; and a filter monitor method from Future Medical Technologies, Inc. (FMTI). The following two critical points in time during the production cycle were selected for sample collection: prior to chick placement in order to determine the resident *Salmonella* population in each poultry house and 1 week before slaughter as an indicator of flock contamination before processing.

MATERIALS AND METHODS

Farm selection and sampling. Fifteen broiler farms belonging to an integrated poultry company in North Carolina were selected to participate in the study. The

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selection criteria included (i) the number of houses on the farm (at least two houses; the sizes of the houses were not considered); (ii) the condition and age of the litter (the houses could not have been cleaned to the ground or the litter could not have been top dressed just prior to placement of the study birds); and (iii) the time of the next bird placement (farms without chicks were scheduled to receive birds within the next 7 to 10 days).

Samples were collected twice during the production cycle, which was approximately 45 days long. The first samples were taken prior to chick placement. The second samples were collected when the birds were about 5 weeks old. A total of eight drag swab samples were collected from two houses on each farm. The houses were divided into four equal quadrants. Each drag swab consisted of gauze pads (8 by 8 in.) that were moistened with 30 ml of buffered peptone water (BPW); the drag swab technique described by Mallinson et al. (12, 13) was used. When sampling was completed, the swabs were placed in a Koolatron transport refrigerator and returned to the laboratory. At the laboratory, the swabs were cut in half aseptically, and one half was placed in a new sterile Whirl-Pak bag. One half of each swab was frozen at -20°C for 3 weeks, and the other half of the swab was placed in a 4°C refrigerator and cultured the following morning.

Sample preparation. The drag swabs (both fresh and frozen samples) were removed from storage, and 50 ml of BPW was added to each Whirl-Pak bag. After the BPW was added, the bags were stomached by hand for 1 min and incubated at 37°C for 4 h for all of the rapid methods except the filtration method. For the filtration method, the samples were incubated for another 4 h. After the 4- or 8-h preenrichment treatment, the bags were stomached again before the samples were used.

Rapid tests. Prior to the start of the project, the manufacturer of each rapid test was given 12 drag swabs collected from a broiler breeder research unit at North Carolina State University to determine whether modifications to the original protocols were needed. Modifications recommended by the manufacturers were incorporated into the study. All of the rapid tests and the standard isolation technique were used for each drag swab collected.

Reveal test. The Neogen Reveal *Salmonella* test is a colloidal gold-labeled antibody ELISA that was developed for use with food samples. The manufacturer reports that a positive or negative result may be obtained in 20 h. In this study, a 1:10 dilution of each sample was preenriched by incubating it in Neogen REVIVE medium (preincubated at 42°C) for 2 to 4 h at 37°C . This medium enhances survival and recovery of injured *Salmonella* cells under stressed conditions. *Salmonella* spp. were then grown to detectable levels by adding the Rappaport-Vassiliadis selective medium provided with the kit. Samples were then incubated for 16 h at 37°C . The sample tubes were vortexed after incubation, and 100 μl of each sample was adsorbed to the ELISA test device. The results were read after 20 min. A positive result consisted of a blue line that developed in the result window. If a faint line was present, the sample was placed in a plastic bag to prevent dehydration. A positive result was recorded if the line became more definite during an additional 20-min incubation period.

BIND assay. The Idetek BIND (bacterial ice nucleation detection) *Salmonella* assay was also specifically designed for use with food samples. This test works by allowing *Salmonella* cells to produce ice nucleation proteins that cause cells to freeze only when the temperature is lowered to -9.3°C or below. Presumptive positive results may be obtained in 22 h (8). In this study, samples were preenriched by incubating them in Rappaport-Vassiliadis medium for 18 h at a 1:100 dilution. After incubation, a 1:10 dilution of each sample in BPW was prepared. The assay and background control tubes were inoculated with 500 μl of each dilution; the background assay tubes were inoculated first. The tubes were vortexed and incubated at room temperature for 2.5 h to allow time for transduction of the ice nucleus phage into the *Salmonella* cells. After incubation, the microtiter trays were inoculated with 50 μl of each sample, placed on a BIND Super-Cooler which had been precooled to -9.3°C , and cooled for 20 min. This resulted in the formation of ice nuclei in the *Salmonella* cells. The results were determined by a color change; red indicated a positive result, and green indicated a negative result. For each positive assay a background assay was performed to check for cross-reactivity with competing bacteria.

Filter monitor method. The FMTI filter monitor method was originally developed for use with liquid samples, such as wastewater from slaughter plants or poultry carcass rinses. According to the manufacturer, this procedure should decrease the time needed for presumptive *Salmonella* colony isolation by 12 to 18 h compared with the traditional culture method (6). This method is very similar to the traditional *Salmonella* culture techniques except that all of the procedures are performed with a single filter monitor apparatus (6). The following two variants of the method are available: sealed filter membrane and rehydrated selective medium pad. This was the only rapid method used in our study which could give quantitative data and grew bacteria to isolation.

In this study, we used three replicates of each sample, two replicates that were diluted 1:10 in BPW and one replicate that was diluted 1:100. One milliliter of a 1:10 dilution and 1 ml of a 1:100 dilution were placed on individual filters which were presealed onto the filtration device surface. One milliliter of a 1:10 dilution was placed onto an unsealed filter which was transferred to a xylose–lysine–tergitol-4 (XLT4) pad after the initial incubation. The appropriate dilution of a sample was added to a filter cup and suctioned through the cellulose grid filter membrane. M-tetrathionate was then suctioned through the filter. Each filter cartridge was incubated for 18 h at 37°C . For the sealed filter replicates, XLT4 broth (supplied with each kit) was added to the filter cups. The unsealed filter

replicate was transferred to a rehydrated XLT4 medium pad. The filters were incubated at 37°C for 8 h. The plates were read quantitatively and qualitatively to determine whether distinct hydrogen sulfide-producing (black) *Salmonella* colonies were present.

Standard culture method. A standard culture method was used as the “gold standard” for comparing the rapid tests. The standard method can take 48 to 72 h to obtain a confirmed diagnosis; using delayed secondary enrichment may add 5 to 7 additional days to this time. Primary enrichment was accomplished by incubating 1 ml of each BPW sample in tetrathionate-Hajna broth at 37°C for 18 to 24 h. Presumptive identification of *Salmonella* spp. was accomplished by culturing the broth on two selective agar media, brilliant green agar with novobiocin and XLT4 agar. Positive colonies on brilliant green agar with novobiocin were pink-white on a red background, while positive colonies on XLT4 agar were black on a red background. The presumptive diagnosis was confirmed by subculturing selected suspect colonies on Trypticase soy agar plates and then testing a single colony with poly-O antisera (Difco Laboratories, Detroit, Mich.).

Spiked samples. To determine the minimum number of CFU necessary to generate a positive result with any of the rapid test methods, four trials were performed with 30 known negative drag swabs per trial (negative as determined by the standard culture method and all three rapid tests). The negative drag swabs were selected randomly, with the researchers were blinded to the selection. Each sample was spiked with a meat and bone meal mixture containing equal numbers of *Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella montevideo*, and *Salmonella senftenberg*. The original spiking mixture was determined by the most-probable-number method to have 10^8 CFU/g. Each trial consisting of 30 swabs was divided into three groups of 10, and each group was inoculated with approximately 10^1 , 10^2 , or 10^3 CFU. The researchers were blinded to each sample's status until after all of the rapid tests had been performed and recorded. Positive and negative controls were included with the samples. The rapid tests and the standard culture technique were performed as described above.

Statistical methods. Contingency tables were developed for the results of each test for fresh and frozen samples to compare each rapid test with the standard isolation method as the gold standard of comparison (5). McNemar's chi-square test (for matched samples) was used to determine whether there were statistically significant differences among the proportions of positive results and negative results for the four tests for fresh and frozen samples at a probability of ≤ 0.05 (16).

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each rapid test for fresh and frozen preplacement and preslaughter samples, as were 95% confidence intervals (5). Sensitivity was defined as the probability of a positive result if a sample was truly positive. Specificity was the probability of a negative result if a sample was truly negative. The PPV was defined as the probability that a sample was positive if that the rapid test was positive, while the NPV was the probability that a sample was negative if that the rapid test was negative.

The sensitivity and specificity of each test were held constant, and the posterior probabilities (the probabilities calculated after reference to the results of the test) were calculated for different prior probabilities of 0.10 to 1.0 in 0.1 increments to generate scenarios for different probabilities. Curves comparing the posterior probabilities and prior probabilities for each rapid test versus the standard test were generated (18).

RESULTS

The results of the rapid tests and the standard isolation method for the preplacement and preslaughter drag swabs (fresh and frozen) are presented in Tables 1 and 2. Preplacement samples from five of the farms had to be discarded due to an incubator malfunction (the sample size decreased from 120 to 80). With the standard isolation method, the *Salmonella* prevalence was 43% for the fresh preplacement samples and 61% for the fresh preslaughter samples.

The Reveal test detected the same number of positive results for the preplacement and preslaughter fresh samples as the standard method detected. However, there was not 100% agreement between the two methods. The Reveal test yielded 10 false-positive results (one was confirmed to be a true positive result by internal culturing) and 10 false-negative results with the preplacement samples. This test also yielded 22 false-positive results and 22 false-negative results with the preslaughter samples. There was no statistically significant difference in prevalence between the Reveal method and the standard method for these samples. The BIND method detected more positive results than the standard culture method detected for fresh samples. There was also no difference in the prevalence of positive results between this test and the standard culture method for the preplacement samples. There

TABLE 1. Rapid test results and standard test results for 80 preplacement fresh and frozen samples^a

Test	Fresh samples (n = 80)				Frozen samples (n = 80)			
	No. of positive results	No. of false-positive results	No. of false-negative results	P value ^b	No. of positive results	No. of false-positive results	No. of false-negative results	P value ^b
Standard	34				10			
FMTI pad	17	2	19	<0.001	10	5	5	>0.05
FMTI 10 ⁻¹ broth	0	0	34	UC ^c	2	1	9	0.027
FMTI 10 ⁻² broth	0	0	34	UC	2	1	9	0.027
Reveal	34	10	10	>0.05	34	27	3	<0.001
BIND	37	14	11	>0.05	25	17	2	0.001

^a All rapid test results were compared to the standard culture method results.^b Determined by McNemar's chi-square test for paired samples.^c UC, unable to calculate.

were 14 false-positive results and 11 false-negative results for the preplacement samples and 37 false-positive results and no false-negative results for the preslaughter samples. The FMTI broth filtration method detected no positive results at either dilution (10⁻¹ and 10⁻²) for the preplacement or preslaughter samples. Many of the filters were overgrown with competitive bacteria, which may have inhibited the H₂S expression of *Salmonella* spp. More favorable results were obtained with the FMTI dehydrated pad filtration method ($P < 0.005$). Two false-positive results were confirmed to be *Salmonella* positive with poly-O antisera. This indicates that the standard method gave false-negative results for these samples. The sensitivities and specificities for all of the tests are presented in Table 3.

The detection of false-negative results by the standard method may lead to false interpretations of the results of the rapid tests. Beckers et al. (1) stated that a false-negative rapid test result and a positive standard isolation result indicate a false-negative rapid test result. However, a positive rapid test result and a negative standard isolation result may not indicate a false-positive rapid test but rather a false-negative standard isolation test (20). Since neither the Reveal test nor the BIND test grows the bacteria to isolation, this cannot be done except by standard isolation from intermediate steps.

When samples were frozen at -20°C for 3 weeks, there was a decrease in the number of positive samples. For the standard method, the *Salmonella* prevalence dropped from 43 to 13% for preplacement samples and from 61 to 23% for preslaughter samples. Since this study was qualitative, a quantitative estimate of the loss in *Salmonella* concentration in each sample was not obtained. The number of false-positive results obtained with the rapid tests increased after the samples were frozen, except for the BIND preslaughter frozen samples. One possible cause of the increase in false-positive results observed with the frozen samples is the possibility that the test reagents may have reacted with antigens from injured or dead *Salmonella* cells. Furthermore, injured cells may not be recovered unless they are repaired prior to selective enrichment and isolation. It is possible that injured cells may not survive in the enrichment broth (4, 11). The results for the Reveal method and the standard method for both preplacement and preslaughter frozen samples differed significantly ($P < 0.001$ and $P < 0.005$, respectively). There was a statistically significant

difference between the BIND method results and the standard method results for the preplacement frozen samples ($P < 0.005$). However, there was no significant difference in the prevalence of positive samples between the BIND method and the standard method for the preslaughter frozen samples. The prevalence of positive samples did not differ between the FMTI pad method and the standard method for the preplacement frozen samples. However, the results of the FMTI pad method and the standard method for the preslaughter frozen samples did differ ($P < 0.01$).

Predictive values are important when the utility of a new *Salmonella* screening test is evaluated since these values are a function of the sensitivity and specificity of the test and the prevalence of disease in the population being tested (15, 18). At a fixed prevalence, a test with high sensitivity tends to have a high NPV, while a test with high specificity tends to have a high PPV.

For the fresh preplacement samples, the PPV ranged from 62% for the BIND method to 88% for the FMTI pad method (Table 4). For the fresh preslaughter samples, the PPV changed from the preplacement sample value, but not consistently in one direction. The PPV decreased for the BIND test and increased for the FMTI pad test but remained essentially the same for the Reveal test. Freezing the samples resulted in a consistent decrease in the PPV for both preplacement and preslaughter samples.

For the fresh preplacement samples, the NPV ranged from 70% for the FMTI pad method to 78% for the Reveal method (Table 4). For the fresh preslaughter samples, the NPV also changed inconsistently from the NPV for the preplacement samples; the NPV for the FMTI pad and Reveal methods decreased, while the NPV for the BIND method increased. When the samples were frozen, the NPV for the FMTI pad method increased for preplacement and preslaughter samples. For the Reveal and BIND methods the NPV increased for frozen preplacement samples, and for the Reveal method the NPV increased for frozen preslaughter samples.

The relationship between prevalence (prior probability) and the probability of a *Salmonella*-positive sample if a rapid test was positive or negative (posterior probability) when the standard culture method was used for both fresh and frozen samples as the reference test was calculated by using Bayes' theorem (10, 18). The sensitivity and specificity of each test were

TABLE 2. Rapid test results and standard test results for 120 preslaughter fresh and frozen samples^a

Test	Fresh samples (n = 120)				Frozen samples (n = 120)			
	No. of positive results	No. of false-positive results	No. of false-negative results	P value ^b	No. of positive results	No. of false-positive results	No. of false-negative results	P value ^b
Standard	73				27			
FMTI pad	22	0	51	<0.001	15	2	14	0.006
FMTI 10 ⁻¹ broth	0	0	73	UC ^c	1	0	26	<0.001
FMTI 10 ⁻² broth	0	0	73	UC	1	0	26	<0.001
Reveal	73	22	22	>0.05	53	39	13	<0.001
BIND	110	37	0	<0.001	37	19	9	>0.05

^a All rapid test results were compared to the standard culture method results.^b Determined by McNemar's chi-square test for paired samples.^c UC, unable to calculate.

TABLE 3. Sensitivity and specificity, with confidence intervals, for rapid methods compared to a standard method

Test	Samples	Prevalence (%)	Sensitivity		Specificity	
			%	95% Confidence interval (%)	%	95% Confidence interval (%)
Reveal	Fresh preplacement	43	71	60–81	78	69–87
	Fresh preslaughter	61	70	61–78	53	44–62
	Frozen preplacement	43	70	60–80	61	51–72
BIND	Frozen preslaughter	44	52	43–61	58	49–67
	Fresh preplacement	46	68	57–78	70	59–80
	Fresh preslaughter	92	100	100–100	21	14–29
FMTI pad	Frozen preplacement	31	80	71–89	76	66–85
	Frozen preslaughter	31	67	58–75	80	72–87
	Fresh preplacement	21	44	33–55	96	91–100
FMTI 10 ⁻¹ broth	Fresh preslaughter	18	30	22–39	100	100–100
	Frozen preplacement	13	50	39–61	99	96–100
	Frozen preslaughter	13	48	39–57	98	95–100
FMTI 10 ⁻² broth	Fresh preplacement	0	ND ^a	ND	ND	ND
	Fresh preslaughter	0	ND	ND	ND	ND
	Frozen preplacement	3	10	3–17	99	96–100
FMTI 10 ⁻² broth	Frozen preslaughter	0	4	0–7	100	100–100
	Fresh preplacement	0	ND	ND	ND	ND
	Fresh preslaughter	0	ND	ND	ND	ND
FMTI 10 ⁻² broth	Frozen preplacement	3	10	3–17	99	96–100
	Frozen preslaughter	0	10	3–17	99	96–100

^a ND, not determined.

kept constant, while the prior probability (prevalence) was changed from 0 to 100% in 10% increments. The results were plotted in order to visualize the relationship between prior probability and posterior probability compared to data for the standard culture method for fresh preplacement and preslaughter samples (data not shown). The tests that produced the lines with the greatest curvature were the tests that provided the most accurate test information. The curves were close to the diagonal, indicating that the tests did not provide as much information as the standard culture method provided (18).

In order to determine the lowest concentration of *Salmonella* cells in a drag swab sample that could be detected by the standard isolation method and the three rapid tests, samples

were spiked with known *Salmonella*-negative drag swabs from the previous rapid test study (Table 5). Except for the FMTI broth at a 10⁻² dilution, *Salmonella* cells were detected by all of the tests at the lowest concentration (10¹ CFU/ml) in at least one of four trials. The BIND and Reveal tests detected *Salmonella* cells at the lowest concentration in all four trials. The standard method recovered the lowest concentration of *Salmonella* cells in two of the four trials. No *Salmonella* cells were recovered from any of the samples spiked with 10² CFU/ml when the XLT4 broth filtration method was used, while the other rapid methods and the standard culture method detected *Salmonella* cells in all four trials at this concentration. The number of isolates was greatest for the standard method at a

TABLE 4. Predictive values, with confidence intervals, for rapid methods compared to a standard method

Test	Samples	PPV		NPV	
		%	95% Confidence interval (%)	%	95% Confidence interval (%)
Reveal	Fresh preplacement	71	60–81	78	69–87
	Fresh preslaughter	70	61–78	53	44–62
	Frozen preplacement	21	12–30	93	88–99
BIND	Frozen preslaughter	26	18–34	81	73–88
	Fresh preplacement	62	51–73	74	65–84
	Fresh preslaughter	66	58–75	100	100–100
FMTI pad	Frozen preplacement	32	22–42	96	92–100
	Frozen preslaughter	49	40–58	89	83–95
	Fresh preplacement	88	81–95	70	60–80
FMTI 10 ⁻¹ broth	Fresh preslaughter	100	100–100	48	39–57
	Frozen preplacement	50	39–61	93	87–99
	Frozen preslaughter	87	80–93	87	80–93
FMTI 10 ⁻² broth	Fresh preplacement	ND ^a	ND	ND	ND
	Fresh preslaughter	ND	ND	ND	ND
	Frozen preplacement	50	39–61	88	81–96
FMTI 10 ⁻² broth	Frozen preslaughter	100	100–100	78	71–86
	Fresh preplacement	ND	ND	ND	ND
	Fresh preslaughter	ND	ND	ND	ND
FMTI 10 ⁻² broth	Frozen preplacement	50	39–61	88	81–96
	Frozen preslaughter	50	39–61	88	81–96

^a ND, not determined.

TABLE 5. Summary of the results of the *Salmonella* rapid test detection limit study

Trial	Spike concn (CFU/ml)	No. of positive samples ^a					
		Standard method	FMTI pad test	FMTI 10 ⁻¹ broth test	FMTI 10 ⁻² broth test	BIND test	Reveal test
1	10 ¹	2	1	1	0	3	1
	10 ²	3	2	0	0	4	3
	10 ³	9	2	1	0	5	3
2	10 ¹	0	0	0	0	1	3
	10 ²	5	2	0	0	4	4
	10 ³	9	3	3	3	4	3
3	10 ¹	2	2	0	0	4	4
	10 ²	3	1	0	0	5	5
	10 ³	8	2	2	1	8	6
4	10 ¹	0	1	0	0	8	5
	10 ²	5	1	0	0	6	6
	10 ³	7	4	0	1	9	4

^a Ten samples per dilution per trial were tested.

concentration of 10³ CFU/ml. The increase in positive results as the *Salmonella* concentration increased was not as strong for the rapid tests as it was for the standard method. No statistical difference between results of the different trials was found. Overall, the Reveal and BIND tests were able to detect *Salmonella* cells at all three concentrations. There were no significant differences in the number of positive results obtained for the three different *Salmonella* concentrations for the two tests. The BIND test did detect *Salmonella* cells in a larger number of samples than the Reveal test detected for all three spike levels in all four trials. The XLT4 broth filtration method did not result in many positive *Salmonella* recoveries at any spike level. A larger number of positive results was obtained with the XLT4 dehydrated pad method, but the number was still considerably less than the number obtained with the standard method. For both methods, competitive growth may have interfered with the recovery of *Salmonella* cells.

The real purpose of using *Salmonella* rapid detection test kits is to decrease the time and labor needed to determine if a sample is positive or negative for *Salmonella* cells. The times needed for each step in the protocols of the rapid tests are presented in Table 6. The time to detection for a negative sample is 1 day less with the Reveal and BIND methods than with the standard method. The filtration method could take 12 h less than the standard method. We found, however, that *Salmonella* colonies did not express H₂S after only 8 h of selective isolation so the filters needed to be incubated overnight to obtain visible black colonies that could be subcultured onto XLT4 agar plates. Approximately 72 h was needed with

all of the tests (rapid and standard) to confirm that a sample was presumptively *Salmonella* positive. This was due to the fact that the enrichment fluids from the presumptively positive rapid tests had to be isolated on selective media and then subcultured onto nonselective media for serologic testing. Time saving occurred only when negative results for samples did not have to be confirmed culturally.

DISCUSSION

With the population of samples tested in this study, all of the rapid tests had very low sensitivities and NPV, which led to many false-negative results. The results of the BIND *Salmonella* and Reveal tests were not statistically different from the results of the standard culture method for the preplacement fresh samples. The Reveal test results were also not different for the preslaughter fresh samples. However, numerous false-positive and false-negative results were obtained with both tests. Only on two occasions was the sensitivity greater than 75% (the BIND test preplacement frozen samples and the BIND test preslaughter fresh samples). Thus, for the population of samples used, the probability that the Reveal or BIND test detected *Salmonella* cells in a truly positive sample was generally less than or equal to 70%. For the Reveal and BIND tests, the specificity was less than or equal to 80% for both sampling periods (fresh and frozen). Sensitivities and specificities can change when the population that is being tested changes. While the *Salmonella* serotypes tested in this study are the same serotypes found on foods for which the tests were designed, the growing conditions from which the bacteria were cultured and the level and type of background contamination were very different.

The PPV decreased overall when the prevalence dropped after the samples were frozen. Likewise, the NPV increased as the prevalence decreased. The curves for the positive prior probability were generally further away from the diagonal than the negative prior probability curves. Overall, the curves remained close to the diagonal for all of the rapid tests. The likelihood of false-negative results with the nonisolation methods at the lower *Salmonella* prevalence was less than the likelihood of false-negative results with the isolation methods. This could lead to problems with confirmation of suspect *Salmonella* rapid test results. Confirmatory isolation methods may give false-negative results for positive rapid test results and could lead to an underestimation of the *Salmonella* status of a flock prior to slaughter.

Freezing the drag swabs at -20°C for 3 weeks strongly affected the rate of recovery of *Salmonella* cells with the standard method, and the number of false-positive results obtained with the Reveal and BIND tests increased. The results actually may not have been false-positive results for the rapid tests; they

TABLE 6. Times required to obtained positive and negative results in all tests

Method	Time required					
	Preenrichment (h)	Enrichment (h)	Testing (min)	Isolation (h)	Subculturing (h)	Total for negative identification (h) ^a
Standard	4	18		24	24	48
FMTI	8	14		8 to overnight ^b	24	36-48
Reveal	4 (+4 in REVIVE)	16	20	24	24	24
BIND	4	18	150 + 20-40 ^c	24	24	24
						72
						48-72
						72
						72

^a Approximate times that do not include the time needed to prepare samples.

^b Overnight if needed.

^c Preparations must be incubated for 150 min at room temperature to allow phage uptake by the *Salmonella* cells.

could have been standard method false-negative results. The Reveal and BIND tests may have detected damaged *Salmonella* cells in the samples that were physiologically active but nonculturable. Any antigen capture system would have the same problems.

The manufacturers of both the BIND test and the Reveal test report that these tests can detect as little as 1 *Salmonella* CFU/25 g of food sample (8, 17). The detection limit for the BIND test after enrichment varies for different *Salmonella* serotypes. It ranges from 10^2 CFU/ml for *S. montevideo* to 10^4 CFU/ml for *S. enteritidis*. Both of these serotypes were present in the spiked material used in the present study. The Reveal and BIND *Salmonella* tests were able to detect *Salmonella* cells at the lowest spike level. There were no significant differences in the number of positive results obtained with the three different levels of *Salmonella* cells for both tests. The BIND test did detect *Salmonella* cells in a larger number of samples than the Reveal test detected for all three spike levels in all four trials. We have not found a detection limit in the literature for the FMTI filtration method. However, two previous reports showed that the filtration method and the standard method did not differ when they were used with broiler carcass rinses or pure *Salmonella* cultures (7, 14). Mallinson et al. (14) evaluated the use of the filtration membrane transfer method with poultry carcass rinses, not environmental drag swabs. Gao et al. (7) found no differences between the broth filtration method and the standard culture method with XLT4 agar. However, the tests were conducted entirely with pure *Salmonella* cultures. Thus, the effect of competitive flora on the recovery of *Salmonella* cells with this method was not evaluated. In the present study, very few isolates were obtained at any spike level with the FMTI filtration broth method in all four trials. The number of positive results was considerably greater with the XLT4 dehydrated pad method. However, there were considerable differences in the numbers of isolates obtained with spike concentrations of 10^2 and 10^3 CFU/ml compared to the numbers obtained with the standard method.

The kits which we used were self-contained; this should be an advantage in an industry setting in which a large number of samples have to be handled at one time. The BIND test was very simple to perform, requiring only routine pipetting to prepare the dilutions and the assay tubes. Reading the test was also straightforward, except that the wells thawed rather quickly. The microtiter tray could be refrozen and the results could be confirmed if they were missed the first time. The Reveal test was easier to use than the BIND test in that the only pipetting necessary was the pipetting to inoculate the enrichment media. Although the test itself was easy to perform, it was often difficult to read because the positive line could be very faint after 20 min. The line sometimes developed further after 30 to 45 min. This made it difficult to label some samples definitely positive or negative. Thus, this test could be subject to reader error. The ability of the Reveal test to strongly detect *Salmonella* cells varies with different serotypes according to the manufacturer. Serotyping of the samples was not done. Therefore, we could not determine if the tests selectively detected certain serotypes. The competitive growth present in the clinical samples may have interfered with the tests and may have led to weak positive results. The filtration method took essentially the same time as the standard method. It was also more laborious initially, with sample dilution preparation combined with filtration of the samples. The 8-h preenrichment time also led to a very long day in the laboratory when many samples were tested at the same time and three filters were used per sample. The filters, especially the broth filters, were also difficult to read. The colonies were often

larger and more defined on the XLT4 pad filters. Environmental samples deposited debris on the surfaces of the filters which could be confused with *Salmonella* colonies.

Based on our results, we do not recommend that the rapid *Salmonella* detection methods which we used (the Reveal, BIND *Salmonella*, and XLT4 broth and dehydrated pad filtration methods), in their present state of development, be utilized with poultry environmental samples collected with drag swabs. Moreover, drag swabs should not be frozen because the *Salmonella* level in relation to the level of the competitive flora is often low when the samples are initially collected and the *Salmonella* cells may be lost when samples are frozen.

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